Quality of Bell pepper (*Capsicum annuum* L.)

affected by drought condition

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Abstract

The climate change is predicted with an increasing number of droughts caused by rising global temperature and limited rainfall, thus generate water scarcity. This condition will be a great challenge for agricultural activities, to produce more resilient fruits and to select adaptable varieties as mitigation to global warming.

Drought condition affects plant growth and production, especially in some crops that very sensitive to water scarcity such as bell pepper. This study aims to investigate the effects of various water stress conditions to bell pepper production and qualities, through limited water supply at half field capacity or constant mild water stress or half field capacity and intermittent severe water stress, compared to control (daily watering in full field capacity). By those different irrigation methods, we assessed plant morphology during vegetative and generative phase, as well as yield and fruit qualities of two bell pepper varieties, Cupra and Viper.

We found that those both varieties in this study were physiologically able to adapt with water stress conditions, but Viper variety had more responsive physiological responses to water stress, showed by reduction on stomatal conductance and photosynthesis rate. Intermittent severe water stress may still appropriate to support the effective use of water (EUW) on both species, and Viper variety expressed better water use efficiency (WUE) by maximizing plant yield under stress. Since genetic-specific adaptation was found in this study, we suggested that the Viper variety had a better drought tolerant mechanism and produced more marketable yield that will benefit small farmers than the Cupra variety.

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CHAPTER I

INTRODUCTION

Pepper (*Capsicum* sp.) is a member of the *Solanaceae* family, which origin is from the tropical Central to South American region (Eshbaugh, 1993). This crop was not only popular for culinary purposes, but also as source of vitamins and minerals (nutritional value), and its medical properties such as antimicrobial and anticancer also been used in medical practices due to its preventive and therapeutic effects to treat cancer, rheumatism, stiff joints, bronchitis and heart arrhythmias. In the ancient times, Aztec utilized pepper as painkiller for aching tooth, chest colds with cough and headache, arthritis, and many other ailments (Bosland & Votava, 2012; Purseglove, Brown, Green, & Robbins, 1979; Saleh, Omer, & Teweldemedhin, 2018).

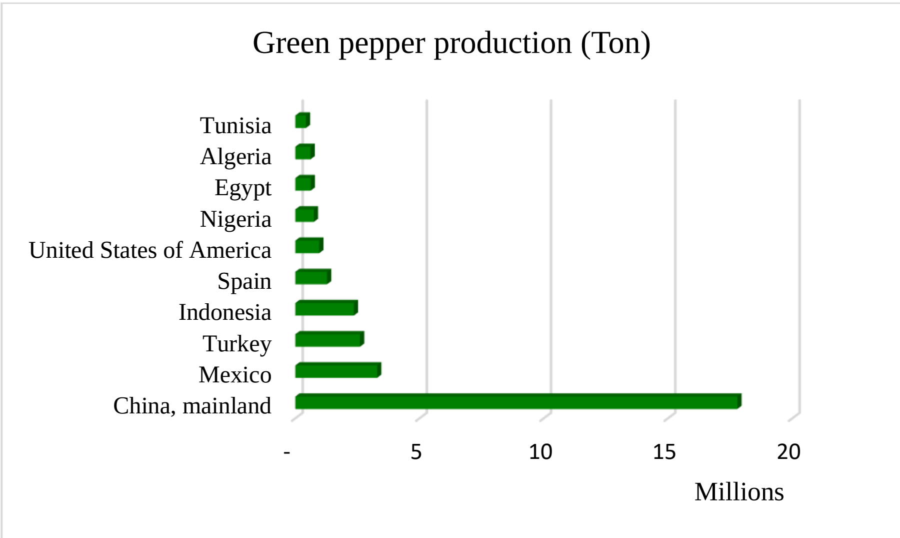


Figure 1. Top 10 World green pepper production (FAOSTAT, 2017).

Peppers were introduced to South East Asia since 16th century, including to Indonesia (Purseglove et al., 1979). Indonesia is the third most population country also rated as the fourth most 'pepper production country' in the world (Figure 1) with number of production near 2,35 million ton (FAOSTAT, 2017). This is due to the high use of peppers in every local meal. According to the Ministry of

Agriculture (2019), there are two main pepper species grown in Indonesia: *Capsicum annuum* (chili) and *Capsicum frutescens* (cayenne). The bell pepper type belongs to *Capsicum annuum* which has blocky shape with sweet taste and it was introduced to Indonesia since early 1990's particularly in West Java (Lina, 2013). However, registered and certified bell pepper seeds were not available until 2004 (Table 1), before that year, they used presumably non-registered seeds. Compared to another chili varieties, prior to 2019, there are only 14 varieties of bell pepper had been registered (4.06%) while new registered varieties had been dominated by chili (75.07%), followed by cayenne varieties (20.8%) Agriculture (2019).

Table 1. Bell pepper varieties registration in Indonesia

No	Variety	Year of registration	Breeder Company
1	Goldflame	2004	PT. Joro Serhalawan
2	Spartacus	2004	PT. Joro Serhalawan
3	Edison	2009	Enza Zaden
4	Suniya	2009	Enza Zaden
5	Inspiration	2010	Rijk Zwaan Seeds Company
6	Taranto	2010	Rijk Zwaan Seeds Company
7	Salomon	2017	PT. Clause Indonesia
8	Solanor	2017	PT. Clause Indonesia
9	Springbox	2017	PT. Clause Indonesia
10	AFN PM 01	2018	PT. Agrofarmaka Nusantara
11	Samshon	2018	PT. Clause Indonesia
12	Deniro	2019	PT. East West Seed Indonesia
13	Spider	2019	PT. East West Seed Indonesia
14	TI 096	2019	PT. Tani Murni Indonesia

(Source: Indonesia horticulture registered variety http://varitas.net/dbvarietas/report/lap4.php)

Indonesia central production of bell peppers are founds in West Java, East Java, Bali and West Nusa Tenggara (BPS, 2017). Although the yearly trend of its production fluctuated (BPS, 2018), there is an increasing demand in specific markets, especially for export and product supply for expansion of the tourist industry (supplying local hotels and restaurants). According to Prabaningrum, Moekasan, Udiarto, den Belder, and Elings (2008), this opportunity creates significant opportunities and encourages farmers to passionately grow and produce high quality bell pepper locally for these markets.

Out of 2.3 million tons Indonesian green pepper production in 2017 (Figure 1), the national bell pepper production contributed only about 1.6% of total chili and cayenne production at the same year (Table 2) Even though if we compare to the previous year, total bell pepper production at this particular year reached a positive growth by 41% (Ministry of Agriculture, 2018; BPS, 2018; Susanti & Waryanto, 2018).

Table 2. Vegetables production in Indonesia 2016 - 2017 (ton).

Commodities	**2016**	**2017**	**Growth**	
			Absolute	**(%)**
Shallot	1,446,860	1,470,155	23,295	1.61
Potato	1,213,038	1,164,738	- 48,300	-3.98
Chilli	1,045,587	1,206,266	160,679	15.37
Cayenne	915,988	1,153,155	237,167	25.89
Tomato	883,233	962,845	79,612	9.01
Carrot	537,521	537,341	- 180	-0.03
Cucumber	430,201	424,917	- 5,284	-1.23
Long beans	388,056	381,185	- 6,871	-1.77
Dog fruit	56,090	66,065	9,975	17.78
Garlic	21,150	19,510	- 1,640	-7.75
Bell pepper	5,254	7,390	2,136	40.65

Source: Ministry of Agriculture (2018).

In addition to small specific markets, it has been suggested that major constraints on bell pepper production in small farmers scale in Indonesia are due to limitation on continuity of supply and the yield quality. This condition may be caused by several factors, among others are lack access of varieties and inadequate technical support on production (Adiyoga, Gunadi, Moekasan, & Subhan., 2007). Yet, lack of postharvest handling may also affect quality (Nyanjage et al., 2005).

Until 2019, all commercial bell pepper varieties in Indonesia are produced by Dutch and French seeds companies. Those varieties essentially set to grow by specific requirements. Controlled greenhouse production systems allow farmers to increase profitability by increasing plant density, decrease of disease, reduction of labour cost and can growing out of season with the guarantee for continuous product supply. However, low budget farmers may grow greenhouse varieties for example in custom-built plastic houses with some limitations to precisely control growing conditions (Gunadi et al., 2008). According to Gunadi et al. (2007), protected cultivation in tropical areas combined with drip irrigation is more profitable for bell pepper cultivation compared to manual watering. Not only to control pest and disease, protected cultivation may be used as a shield from heavy rain in tropical countries as well.

A lot of research have been done to improve commercial pepper production for example nitrogen use efficiency with drip irrigation on pepper production (Yasuor, Ben-Gal, Yermiyahu, Beit-Yannai, & Cohen, 2013) while increasing nitrogen input up to 227 kg/ha was necessary to yield maximal production (Zhang, Liu, Tan, Hong, & Warner, 2010), Plant resistance to pest and disease development also investigated due to shading (Díaz-Pérez, 2014), genetic trait by QTL (Maharijaya et al., 2015), or by utilization of natural enemies (Prabaningrum et al., 2008). Attention on improvement on fruit quality aspect in greenhouse (Gruda, 2005; Jovicich, Cantliffe, Stoffella, & Haman, 2007) were also been studied.

It is necessary to conduct study on quality of marketable fruit especially for bell pepper under limited water condition to anticipate climate change. High quality of bell pepper is possible in the dry season providing adequate irrigation is available; but irrigation system can fail by decreasing on water supply during prolonged and very dry conditions with high evapotranspiration in extremely hot tropical climates.

The climate change is predicted with an increasing number of droughts caused by rising global temperature, intermittent rain and this will likely generate water scarcity, affecting the horticulture product. These conditions will bring a challenge to produce more resilient fruit to small farmers. Selecting the new varieties with different abilities to respond to insufficient moisture conditions which can be by intensity, duration, frequency and/or lack of rainfall.

Maintaining the quality of the fruit attracting higher prices can be a challenge during scarcity of water. Many such attempts had successful results for example Kirnak, Tas, Kaya, and Higgs (2002) found that marketable eggplant was reduced 12% with 20.4% water saving; watering around 75% ET_c (potential crop evapotranspiration) on tomato tropical greenhouse farming giving the optimum fruit on Troy 489 variety (Harmanto, Salokhe, Babel, & Tantau, 2005). However, to save 50% from normal irrigation water will reduced the dry mass of tomato by 23% (Zegbe, Behboudian, & Clothier, 2006) and mulching will help to mitigates negative effect of water stress on plant growth and fruit yield in field grown pepper (Kirnak, Kaya, Higgs, & Tas, 2003) by holding soil water and reducing evaporation from soil.

This thesis will describe the responses of bell pepper varieties to a variety of watering conditions in controlled cultivation under glass-house conditions at the Massey University Horticultural Unit (Palmerston North, New Zealand). The vegetative growth, physiological responses, yield and harvest quality that observed at different treatment on two different bell pepper varieties are discussed here. The information gained from this study will provide a recommendation to develop further trials on specific water scarcity conditions, especially to be adapted in hot climates and limited water supply areas in an Indonesian context.

CHAPTER II

LITERATURE REVIEW

2.1 General information about *Capsicum*

Among of 38 species, there only five pepper species are domesticated and cultivated: *Capsicum annuum, C. frustescens, C. chinense, C. baccatum,* and *C. pubescens,* with common or local name as described in Table 3 (Bosland & Votava, 2012). *C. annuum* is the most extensively cultivated species, compared with the other four cultivated pepper species (Pickersgill, 1997). This species is also known to have the greatest economic importance since it presents a largest distribution worldwide. *C. annuum* is usually consumed either raw or cooked and used as additive in the food industry (Pino, Sauri, & Marbot, 2006). Moreover, *C. annuum* varieties are also distinguished by fruit shapes and size, which are specified as chili, bell pepper, paprika, capsicum, jalapenos, cayenne peppers (Bosland & Votava, 2012).

Table 3. Five domesticated Capsicum species and common name for varieties in each species (Bosland & Votava, 2012).

Species	Common Name
C. annuum	Bell pepper, Cayenne, Chiltepin, Jalapeno and Paprika
C. baccatum	Aji and brown pepper
C. chinense	Datil, Habanero and Scotch bonnet, Trinidad Scorpion
C. frutescens	Bird's eye, Tabasco, Melagueta
C. pubescens	Rocoto, Quechuan, Tree pepper

In term of taste and spiciness, *C. annuum* is divided into two groups, pungent and non-pungent, or also called as hot and sweet pepper. Spiciness and pungent level in capsicum is controlled by secondary metabolite compound from alkaloid group called *capsaicinoids* which only found in the genus Capsicum (Collins, Wasmund,

& Bosland, 1995). Spiciness is quantified with Scoville scale heat units. Based on this unit, the level of spiciness in chillies and peppers were classified as below in Table 4.

Table 4. Scoville heat units in type of chillies and peppers (Welbaum., 2015).

Type of chillies and peppers	Scoville heat units
Bell pepper, Sweet pepper, Cubanelle	0
Coronado, Pepperoncini, Pimento	100 - 1,000
Anco	1,000 – 2,000
Guajillo pepper, Jalapeño, Chipotle	3,500 - 10,000
Serrano pepper, Peperoncino, Morita	10,000 - 30,000
Cayenne pepper, Tabasco pepper	30,000 - 50,000
Thai chili, Tepin	50,000 - 100,000
Habanero, Scotch bonnet, Bird's eye chili	100,000 - 350,000
Red savina habanero	350,000 - 580,000
Trinidad moruga scorpion, Naga Viper pepper, Bhut jolokia (ghost pepper), Carolina reaper	580,000 - 2,200,000*)
Police grade pepper spray	5,300,000
Pure capsaicin	16,000,000
Resiniferatoxin	16,000,000,000

*) source: *https://www.cayennediane.com/hottest-peppers/*

2.2 Cultivation of bell pepper

Capsicum annum L. consists of different varieties and the most commonly used in greenhouse production is the bell shaped hybrid, which is commonly known as bell pepper. Basically, small farmers grown bell pepper in the field to supply local markets. While big growers in commercial scale usually grow this plant in greenhouses with precise irrigation systems and fertigation management to produce high export quality fruits. Bell pepper can be produced on a wide range of soil types (Kelley & Boyhan, 2009), but they grow best, however, in deep, medium textured sandy loam or loamy, fertile, well-drained soils. Bell pepper is considered to be moderately deep rooted (Kelley & Boyhan, 2009) and are usually transplanted into plastic mulch on raised beds (Calpas, 2004; Romic, Romic, Borosic, & Poljak, 2003). A raised bed retains warmth and enhances early growth, as well as reducing the risk of waterlogging (Kelley & Boyhan, 2009).

Optimal plant population per hectare depends on plant growth habit. Bell pepper types are more compact than other kinds of pepper (Kelley & Boyhan, 2009) and traditional plastic mulch production planted on a bed of about 35-45 cm width, with plants spaced 30 cm with two rows on each bed (Kelley & Boyhan, 2009). According to (Russo, 1991), population densities up to 11.1 plants m^{-2} can increase plants producing fewer, but similar sized, fruit. Bell pepper need more or less 75 days from transplanting to first harvest (Weiss, 2002). Although being a perennial, bell pepper is grown as an annual in temperate climate and yield is harvested continuously for several weeks (Calpas, 2004).

Vegetable greenhouse production is preferable due to better control of the product quality (high visual quality) and yield (high marketable value), compare with yield from field production. Crop cultivation in greenhouse able to create nearly optimal condition for plant growth and development during unfavourable climate condition such as severe (extreme dry) or mild climate during cold winter climate with low sun intensity (Gruda, 2005). In bell pepper, control on microclimate condition inside the greenhouse may provide optimum growing conditions to achieved better plant growth and development (Jovicich, Cantliffe, & Stoffella, 2004).

To some extent, growing indeterminate bell pepper varieties in greenhouse requires constant pruning (Jovicich et al., 2004), because the indeterminate type will continually grow new stems, leaves and will continuous bloom, set new fruit and ripen fruit throughout the season. There are two ordinary planting system to be used, V trellis and Spanish trellis system (Jovicich, Cantliffe, & Stoffella, 2003b). The V trellis system is used by Canadian and Dutch greenhouse growers. This system consists of forming a two-stem plant by removing one of the two shoots that develop at each node. A twine is used to keep two pair of stems hanging vertically as they grow as showed in Figure 2 below.

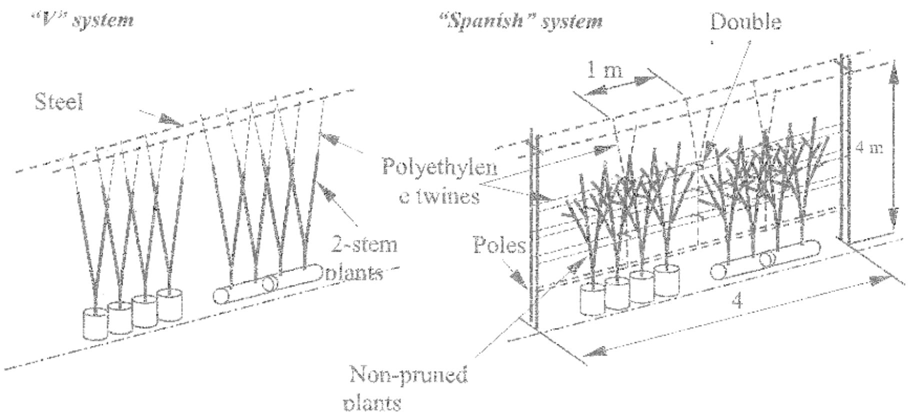


Figure 2. Plant trellised with: "V" system and the Spanish system (adapted from: Jovicich, et al. (2003b)).

According to Jovicich, Cantliffe, and Stoffella (2003b), plant trellised to the Spanish system, the stem and the lateral branches are not pruned and leave the canopy development with 2 to 4 main stems. The row of plant canopies is supported vertically by horizontal twines on both sides, attached to the poles along the plant row. Further research (Jovicich et al., 2004) showed that pruned bell pepper produced 50% fewer flower buds supporting nodes than non-pruned plants, but had a greater percentage of fruit set. In regards of trellis systems, fruit set per plant decreased linearly as plant density increased and Spanish trellis system at density 3.8 plants/m^2 resulted in greater yields of extra-large fruit and required 75% less labour than the V system to prune and support the plant canopy (Jovicich et al., 2004). Jovicich, Cantliffe, and Stoffella (2003b) and Jovicich et al. (2004) also mentioned that the trellis systems did not affect total marketable fruit yields but production of extra-large fruit was higher (38%) in non-pruned than in pruned plants. Moreover, those reports mentioned that non-pruned plants had more marketable yield because it produced lower blossom end rot fruits (32% blossom end rot) compared with pruned plants (62% blossom end rot).

Blossom end rot (BER) is one of the common disorders which causes huge loss for bell pepper production (Hochmuth & Hochmuth, 2009). The disorder emergence from Calcium (Ca) deficiency (Gruda, 2005). There are two type of mechanisms which cause the deficiency. The first is a low concentration of Ca availability on the media which caused insufficient supply for the tissue during fruit development. The second is the inability of the plant itself to supply sufficient Ca due to the fast-growing fruit tissue. According to Hochmuth and Hochmuth (2009), some factors can increase the BER incidence like: inordinate nitrogen fertilization, excess fertilization and inadequate irrigation. Marcelis and Ho (1999), stated that higher temperature and radiation will create more Ca flow to leaves and reduce the Ca supply into a fruit which may cause additional BER issues.

There are some ways to prevent BER incidence like to have a good irrigation system and nitrogen fertilizer management, soil with no shallow compaction for better bell pepper rooting system (Hochmuth & Hochmuth, 2009) and manipulate the root function by using polyethylene mulches (Simonne et al., 2006).

2.3 Some aspects that determine bell pepper quality

According to Camelo (2004), the word "quality" comes from the Latin *qualitas* and it has meaning as an attribute, or basic nature of an object. Kader (2002), defined quality as the "degree of excellence or superiority". It can be said that a product is of better quality when it is superior in one or several attributes that are objectively or subjectively valued. The different criteria can be used for judging quality for the same crops, it depends on the objective.

Quality of horticultural products immediately affect the market value. According to Maalekuu, Elkind, Tuvia-Alkalai, Shalom, & Fallik (2004), market value of bell pepper is determined by the visual appearance including the absence of decay, insect infestation or mechanical injury, also fruit size, colour, firmness, crispiness and flavour. Marketable fruit are normally weighed, counted, and graded by size following a diameter scale used for imported greenhouse-grown bell peppers: extra-large [diameter >84.0 mm], large [76.0 to 83.9 mm], medium [64.0 to 75.9 mm], and small [56.0 to 63.9 mm]. According to Jovicich et al., (2007), fruit with blossom end rot, flat-shaped fruit, and fruit with diameter smaller than 55.9mm is unmarketable.

There are complex conditions that can reduce plant productivity and quality, during growth and development process, which are influenced by internal and external factors. For internal factors, it is mainly determined by genetic composition, while for external factors is mainly the variation of the environment.

Some environmental aspects are generally known to influence bell pepper quality, such as temperature, irradiation, humidity, nutrition and the availability of water. Sun light intensity, adequate water supply and relatively moist soils are required during the total growing period to produce high yield and high quality bell pepper (Madramootoo & Rigby, 1991). Bell pepper plants can be productive in temporary suboptimal environments, but high-quality coloured fruit can be reduced as a result of physiological disorders such as cracking on fruit walls (russet and radial scars), yellow spots, and necrosis on the blossom end of the fruit (Aloni, Pressman, & Karni, 1999; Marcelis & Ho, 1999).

Bell pepper requires warm temperature to grow well, but fruits are very sensitive to sunburn. This plant growing well in daytime temperature between 22-28°C and night around 18^{o}C. Increasing daytime as well as night-time temperatures increased flower and fruit abortion, especially at higher night temperature (Rylski & Spigelman, 1982). On the contrary, low temperature affects fruit quality which result in small and flattened fruit, and usually produced a parthenocarpy fruits (Aloni et al., 1999), with cracking and pigmentation on the fruit skin (Rylski, 1986).

There are some factors that influence yield quality in bell pepper: i) climatic conditions, ii) cultural practices, iii) maturity at harvest, iv) harvesting method and v) postharvest handling procedures. In Florida, USA, high quality bell pepper fruits were able to be produced in soilless media irrigated with a complete nutrient solution (fertigation), inside passive ventilated greenhouse (Jovicich, Cantliffe, Stoffella, & Vansickle, 2003a). According to these authors, quantity and timing of delivery of water nutrients affect water availability to plants and directly affect fruit yield, fruit quality and production cost. An input of half concentration on nutrient solution per day will decrease a number of extra-large fruit.

2.4 Environmental stress condition: plant response and adaptation mechanism to water deficit

Drought conditions related to water uptake is a potential cause that affects plant growth and development, and therefore production. Especially in bell pepper, since this crop production is very sensitive to water scarcity (Boyer, 1982) and also known as one of the most sensitive horticulture crops to drought stress (González-Dugo, Orgaz, & Fereres, 2007). According to Katerji, Hamdy, Raad, and Mastrorilli (1991), water shortage is particularly detrimental when occurring during flowering or fruit set. Water stress in these periods modifies canopy structure and plant size, causing a reduced leaf area and increased leaf density.

According to Jones (2013), plants adapt to water stress conditions through three major physiological mechanisms: i) avoidance of plant water deficit, ii) tolerance of plant water deficit, and iii) efficiency mechanisms. Plants avoid water deficit with some mechanisms: by developing drought escape mechanism (for examples: shortening growth cycle, developing dormant period during dry season); developing water conservation strategies by producing small leaves, decreasing leaf area, and stomatal enclosure; and maximising water uptake by developing a good rooting system.

Further plant physiological adaptation to water stress condition is developing tolerant mechanisms through osmotic adaptation (turgor maintenance) and production of intercellular compatible solutes. Lastly, plants adapted with the effective use of available water and maximising water consumption by developing particular mechanism such as stomatal enclosure (especially in the afternoon) and developing high proportion of dry matter in seed.

At moderate water stress, plants developing a tolerant mechanism through stomatal enclosure, which then diminished leaf carbon fixation (Chaves & Oliveira, 2004) and shoot growth was reduced as affected by changes in turgor pressure (Hsiao &

Acevedo, 1974). Some plants may also developing drought escape strategies by slowing growth and redirect assimilates and energy to develop plant adaptation by producing protective molecules to fight stress (Zhu, 2002) and/or to maintain root growth and improve water acquisition (Chaves, Maroco, & Pereira, 2003). Osmotic adjustment (OA) is "a biochemical mechanism that helps plants to acclimatize to dry and saline conditions" (Sanders & Arndt, 2012) is also counted as a major cellular stress adaptive response in certain crop plants that enhances dehydration avoidance and maintains yield under stress (Blum, 2005). To be more detailed, Sanders and Arndt (2012) explained that osmotic adjustment is needed by plants to survive by increasing number of active osmotic substances in the cell, which lead to improve degree of cell hydration and maintain plant metabolisms, i.e. to help to protect cellular proteins, enzymes, and cellular membranes against dehydration. Active osmotic substances are included inorganic cations and anions, organic acids, carbohydrates, and amino acids, as well as some protectant solutions such as sugars, cyclitols, proline and glycine betaine. Those protective solutions accumulate in the cytoplasm.

Water requirement of *Capsicums* varies from 600 mm to 1250 mm per growth cycle depending on climate, soil environment, region and variety (Bahadur, Chatterjee, Kumar, Singh, & Naik, 2011; Doorenbos & Kassam, 1979; Richards, 2006). Since genetic variability plays an important role in plant adaptation, we considered that genetic-specific differences between bell pepper varieties also possesses different physiological adaptation mechanisms under stress, as well as in water scarcity conditions. In order to maintain productivity in deficit water condition, in particular drought tolerance in bell pepper, there is a need to understand the plant physiological response during water stress. Comparing how varieties adapt to water stress was the topic of this study to promote our understanding of 'successful' strategies available to bell peppers.

2.5 Bell pepper response in drought condition

Capsicum yield and fruit quality is very sensitive to water stress throughout the growing period, and has been classified as (very) susceptible to water deficit especially at flowering stage (Sezen, Yazar, & Eker, 2006) until fruit development stages (Katerji, Mastrorilli, & Hamdy, 1993). High quality bell pepper production is possible in the dry season by providing adequate irrigation (Doorenbos & Kassam, 1979) while low availability of water will impact on bell pepper production.

Plant water deficits may occur as a consequence of seasonal decline in soil water availability, developing in the long term, or may result from short drought spells (Chaves & Oliveira, 2004). There are numerous ways on plant strategies to control water status and resistant to drought (Schulze, 1986). Slower growth has been suggested as an adaptive feature for plant survival under stress because it allows plants to divert assimilates and energy. Instead of using for shoot growth, plant develop protective molecules to fight stress such as osmotic stress-activated protein kinases (SOS1, SOS2, SOS3) and increasing levels of abscisic acid (ABA) during stress conditions (Zhu, 2002), As a physical response, plants develop strategies by maintaining root growth for improved water acquisition (Chaves et al., 2003).

In general, locally adaptive varieties from climates with marked seasonality were able to better acclimate to the fluctuating environmental conditions, enhancing their efficiency for those conditions. In the case of slowly developing water deficits, plants may also escape dehydration by shortening their life cycle (Chaves & Oliveira, 2004). To this concern, agronomists are improving cultural practices and breeders introducing crop genotypes from drought-prone areas. Moreover, understanding the mechanisms behind drought resistance and the efficient use of water by the plants is fundamental to be able to improve production by developing new varieties.

Reduction in water supply during the growing period in general has an adverse effect on yield and the greatest reduction in yield occurs when there is a continuous water shortage until time of first picking. It was found in bell pepper varieties, that drought stress during plant growth and development stages resulted in reducing leaf area and decreasing both fresh and dry weight but did not affect flower proportion to set fruits (González-Dugo et al., 2007).

Tardieu, (2013) described a detailed plant physiological response and adaptation to deficit water stress conditions. The most sensitive parts to water deficits in plant are in leaves, internodes and reproductive organs. Thus, plant signalling water shortage to those organs by reducing activities on growth and tissue enlargement, which normally those two activities consume a lot of water. Prolong water stress will promote abscisic acid (ABA) production, a plant hormone which regulate further mechanism for stomata closure. This physical response is one of plant adaptation strategy to reduce water loss in tissue. Hereafter, plant aims to deposit available soil water by reducing leaf growth rate and improve leaf water status. This activity also benefits to reduce the rate of photosynthesis and reducing leaf temperature. After this point, further water deficit will accelerate senescence on plant, thus reduce crop cycle duration. Plant utilise limited water available to complete its cycle by redirecting assimilates to the reproductive organs and producing fruits.

According to Tardieu (2013) those plant responses mentioned are considered as plant adaptive strategies to survive with limited water available. He described that during initial water scarcity, the plant tried to escape ("an escape strategy") from stress by reducing water consumption and evaporation demand. By this way, plants also reduce photosynthesis process to avoid severe terminal stress. In further limited water conditions, plants already in intermediate stress situation and will do an avoidance strategy to maintain transpiration rate. Thus, hydraulic conductance is applied to regulate or improve root transfer and water uptake system. Plants also reduce leaves transpiration by stomatal closure, as well as reduce leaf area. The last two adaptive mechanisms (stomatal closure and decreasing leaf growth rate) were also known as plant evolution to survive, by reducing water demand and anticipating the risk of failure to reach the end of the growing season.

There are also some studies discussing the effect of water stress at various stages of plant growth and development, under several stress periods or methods in pepper. Observation of drought stress at vegetative and generative stage of this species was shown that plant endure water stress at seedling stage, and become more harmful at mature stage (Ferrara, Lovelli, Tomasso, & Perniola, 2011). Moreover, extended water stress period at seedling stage was more detrimental than at generative development, which generate flower loss, fewer and poorer fruits quality (Ferrara et al., 2011; Techawongstien, Nawata, & Shigenaga, 1992b).

According to Fernández et al. (2005), plants started to express water stress at day 80 after planting with crop evapotranspiration to water stress at a threshold value 55% of available water content, while soil water uptake was contributed around either 20% (watering at 50% of plant requirement) or 43-47% (watering at 20% of requirement) to water stress. Thus, Fernandez et al., (2005) highlights that drought condition did not significantly affect number of total fruits, but substantially increased the proportion of unmarketable pepper fruits due to their small fruit size, and to high incidences of sunburn and blossom-end rot.

There was significant reduction in total yield of various crops due to drought condition that drove some physiological changes during stress. A better understanding of the mechanisms associated with drought tolerance might be explained by early recovery of the plant physiological conditions, for examples leaf water potential, leaf respiration, leaf chlorophyll content, photosynthetic rate and stomatal conductance, which positively correlated to harvest or fruit yield (Ferrara et al., 2011; Okunlola, Olatunji, Akinwale, Tariq, & Adelusi, 2017; Techawongstien, Nawata, & Shigenaga, 1992a).

Pepper plant responses to water stress have been widely reported. According to Katerji et al. (1991), water stress significantly reduced number of flowers per plant, size, number and fruit weight. Furthermore, it was found to obstruct cell wall development in bell pepper fruits due to reduction in calcium uptake, which causing BER (Ferrara et al., 2011). On the contrary, Katerji et al. (1993) found that early flowering and fruit setting as the most sensitive response to water stress. Moreover, genetic background (Thai chilli vs New Ace pepper cultivars) or adaptability also influences plant response to water stress (Sato, Moreshet, Takagaki, Shinohara, & Ito, 2003).

Longer gradual reduction of water supply was more unfavourably for plant growth and development, compare to sudden drought condition by completely withholding water supply for a short duration (Techawongstien, Nawata, & Shigenaga, 1992b).

Furthermore, enhancing water stress in several intervals (holding back watering at day 74-75 after seeding and day 78-79 after seeding) induced physiological plant tolerance to stress compare to delay and sudden stress (without irrigation at day 78 to 79 after seeding) (Sato et al., 2003). Genetic variability leads to variability in water stress tolerance amongst varieties. Comparing how varieties respond to water stress will therefore promote our understanding of 'successful' strategies available to bell peppers: such as deep rootedness, or reduced leaf size, or stomatal closure, or accumulation of compatible solutes.

2.6 Efficient irrigation management system

Value of water will definitely go up in the future due to severe competition for water from human activities, intensive agriculture, flora and fauna, and their ecological niches (Bouwer, 2000). Proper irrigation scheduling and techniques is required for maximizing yield and effective water use.

Reduction in water supply during the growing period in general has an adverse effect on yield and the greatest reduction in yield occurs when there is a continuous water shortage until the time of first picking (Sezen et al., 2006). Continuous water stress throughout the season can diminish leaf area, fresh and dry weight, but did not hasten ripening, necessary for mechanical harvest, but rather delayed fruit maturation in relation to other treatments (Schnitzler, Sharma, Gruda, & Heuberger, 2004).

Past research and practical experience has shown that irrigation management practices must be simplistic, robust, useable and flexible within the existing system design and maintenance constraints, and understandable by growers, in order for them to be widely adopted and used (Gruda & Tanny, 2014). Product quality, on the other hand, is a complex issue not only depending on different factors. Efficient use of water by irrigation is therefore become increasingly important, and alternative water application methods such as drip and sprinkler irrigation, may contribute substantially to the best use of water for agriculture and improving irrigation efficiency (Sezen et al., 2006).

The effective use of water (EUW) is defined as "plant production under most conditions of limited water supply" (Blum, 2009), EUW indicates the maximal soil moisture consumed for transpiration that expressed in plant production. On the other hand, a new concept, water use efficiency (WEU), is also considered as an important determinant to plant yield under stress. According to Tardieu (2013), WUE is defined as "the ratio of the biomass accumulated on one day to the transpiration rate on the same day". WUE is regulated mainly by plant traits. Here, during photosynthesis, high evaporative demands will increase transpiration, thus decrease WUE value. By those two concepts, new irrigation strategies; regulated deficit irrigation by reducing water supply or partial root drying by withholding water for certain period, for instance, improve the potency to maximize the use of water by allowing crops to withstand under constant mild water stress with less marginal decreases of yield and quality. Moreover, drip irrigation and protected cultivation allegedly improve water use efficiency (WUE) by reducing run off and evapotranspiration losses.

According to (Díaz-Pérez, 2014), reducing water irrigation to 70%, 67%, 50% ETc with watering every 2-3 days since week 5 (after transplanting to the field) responded to maximize vegetative growth and produced similar fruit yield to 100% ETc. However, leaf net photosynthesis and stomatal conductance were reduced and incidence of BER were increase by reduction in ETc to 67% or below. While reducing irrigation to severe water stress status at 33% ETc increased incidence of BER, fruit soluble solid and affected fruit quality.

It is now recognized that fine-tuning irrigation can improve crop efficient use of water, allowing more precise use of water and, at the same time, having a positive impact on the quality of the products. Moreover, plants are commonly subjected to multiple stresses in addition to drought, such as high light and heat under field conditions. When water deficits become too intense or to be in the range of leaf RWC lower than 70%, or too prolonged, leaves can wilt, cells shrink, and mechanical stress on membranes may follow (Chaves & Oliveira, 2004).

Recovery under drought conditions is closely linked to plant capacity to avoid or to repair membrane damage, maintaining membrane stability during dehydration and rehydration processes (Chaves & Oliveira, 2004). Most of the terrestrial plants have evolved either to escape drought by appropriate phenology or to avoid drought, by developing strategies that conserve water or optimize water acquisition. This requires early warning systems and different types of signalling (Chaves & Oliveira, 2004).

Irrigation and fertilization can be managed to minimize the occurrence of fruit disorders and to maximize the marketability of the produce. High frequency of irrigation is recommended only if yield of first-quality fruit could be increased, and if large volumes of water and amounts of nutrients drained from the plant containers could be recycled into the same or another crop (Jovicich et al., 2007). Moreover, an increase of yield of first-quality fruit might be possible using low-cost technologies that can modify the environment to avoid extremes in low and high temperature, radiation, and humidity that are conducive to fruit disorders, such as a single polyethylene layer in the roof and screen systems, temperature-activated side curtains, and heating systems near the plant container (Jovicich et al., 2007).

Deficit irrigation is a common practice to cope with limited water availability (Argyrokastritis, Papastylianou, & Alexandris, 2015). Before implementing a deficit irrigation programme, it is necessary to know crop yield responses to water stress, either during defined growth stages or through the whole season (Kirda, 2002). High yielding varieties are more sensitive to water stress than low-yielding varieties (Kirda, 2002).

Deficit irrigation is a strategy, which allows a crop to sustain some degree of water deficit in order to reduce irrigation costs and potentially increase revenues. In deficit irrigation, the application of irrigation below the full crop evapotranspiration is potentially able to improve efficiency and maximize profits through a reduction in capital and operating costs (Capra, Consoli, & Scicolone, 2008). Thus, deficit irrigation is an optimization strategy whereby net returns are maximized by reducing the amount of irrigation water applied to a crop to a level that results in optimal yield production with a minimum rate of water application.

Under deficit irrigation practices, common agronomic practices and crop husbandry may require modification. However, stress applied during reproductive growth can affect fruit or grain set, resulting in decreased yields. The effects of stress on yields are complex and may differ with species, cultivar and growth stage. Crops or crop varieties that are most suitable for deficit irrigation are those with a short growing season and are tolerant of drought. In order to ensure successful deficit irrigation, it is necessary to consider the water retention capacity of the soil and successful deficit irrigation is more probable in finely textured soils (Kirda, 2002)

Deficit irrigation application has been proved a successful strategy to promote growth of many crops grown. However, the main concern is the need to convince farmers and irrigation practitioners not only of the economic value of deficit irrigation, but also of its practicality (Brugere & Lingard, 2003; Lecler, 1998). According to Kader (2002), the key for growers to adopt appropriate cultural practices is encouraged by the willingness of consumers to pay a premium price for preferred products, essentially compensating the producer for the loss in yield (Gruda & Tanny, 2014).

High value agricultural product (HVAP) may have higher value-to-weight ratio, than high volume commodities. They are often associated with higher investment and risk option than field crops. Generally supported by more intensive production system in terms of land area and labour requirements.

HVAP are often differentiated from lower value goods due to their perishability, scarcity, historical and cultural significance or difficulty in either production or delivery at quality market. Higher returns are achieved because these products possess attributes for which the consumers are willing to pay premium prices. Among these attributes, some can be inherent, such as the content of particular substances: stimulants, aromatic, medicinal properties, micronutrients, vitamin, antioxidants etc. HVAPs can only benefit the poor when they fit into the existing farming or eco-system, making use of the available labour in times when not otherwise employed (FAO, 2005).

This study aims to investigate the effects of water scheduling and different method of irrigation on crop morphology, dry matter, yield and qualities of some bell pepper varieties. Moreover, further results from this study are expected to be examined in larger field experiments to develop high value agricultural product (HVAP) to benefit small farmers in developing countries.

The specific aims or objectives of this study are:

1. To compare and investigate the effect of deficit water conditions (withholding water into severe water stress condition and reducing water supply into half field capacity) to plant vegetative growth, yield and quality of two bell pepper varieties;
2. To investigate any physiological or yield (fruits production and quality) between species in response to water stress conditions (withholding water into severe condition and reducing water supply into half field capacity).

CHAPTER III

Material and Methods

3.1 Growth Conditions

A glasshouse experiment was carried out from September 2017 to March 2018 at Horticultural Unit of Massey University Palmerston North, New Zealand. Two bell pepper (*Capsicum annuum*) varieties, Cupra and Viper (South Pacific Seeds New Zealand) were used as two commercial varieties in this trial. Seeds were sown in Cultilene germination trays containing 2 cm x 2 cm of rockwool cylinder and covered with thin layer of vermiculate (Figure 3). The trays were positioned on the ebb and flow system with automatic watering system every 9 am and 3 pm for 15 minutes. While in the germination glasshouse, the temperature was set on range 15° - 26°C. The heating system turned on when temperature fell below 15° and the fans will work when the temperature rose above 26°C. The nutrient solution EC level is 1.0 with pH 7.5.

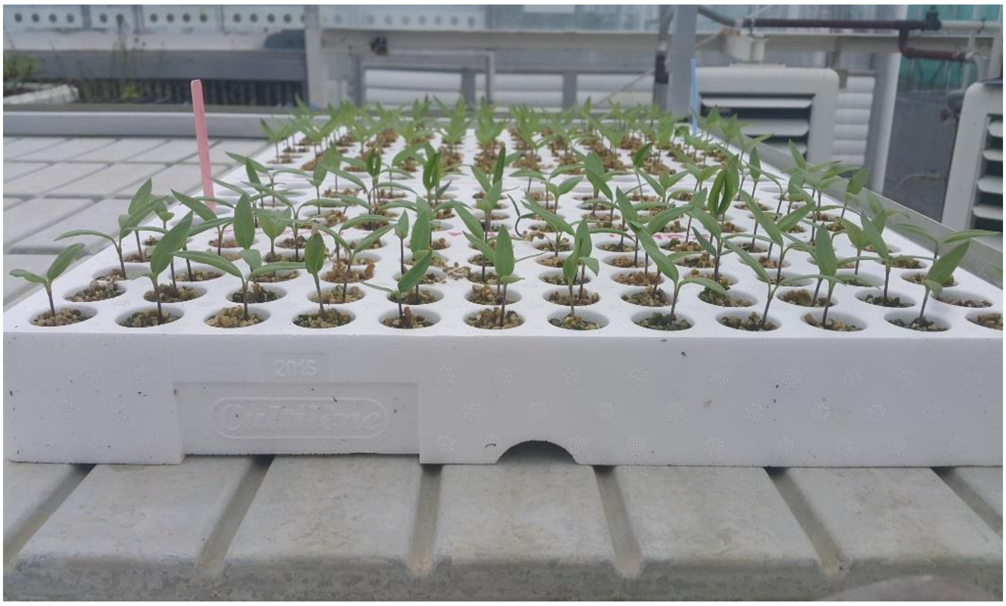


Figure 3. Bell pepper germination 14 days after sowing.

3.2 Transplanting

Seven weeks old seedlings were transplanted into 10 litre rigid pots and grown in a 7 m by 15 m glasshouse on 1 November 2017 (Figure 4). The glasshouse was heated when temperature fell below 15°C and ventilated by fans when the temperature rose above 26°C. Bark fibre and bark fines media was mixed with long-term fertilizer N: P: K; 18: 2.2: 8.3 and Ezyspread dolomite Ca (21%) and Mg (10%). All-rounder solution, N: P: K; 20:20:20 (Peter's Professional, ICL specialty fertilizers, NZ) was applied manually as fertilizer twice a week from transplanting to flowering. Start from flowering time, the application of fertilizer solution was applied automatically using a Dosatron blossom booster, N: P: K; 10: 30: 20 (Peter's Professional, ICL specialty fertilizers, NZ).

To know exactly the water capacity from media, field capacity from media was checked with saturated five filling pots and then the excess water drained for two days. The reading was average from five measurements using time domain reflectometry (TDR).

The drip irrigation system was set up using 2l/h and 4l/h dripper (CETA©, Antelco, NZ) Irrigation was automatically scheduled for seven times a day starting from 6.00 am to 6.00 pm (6.00 am, 8.00 am, 10.00 am, 12.00 pm, 2.00 pm, 4.00 pm and 6.00 pm) with four minutes duration for every run.

3.3 Treatments

Three irrigation treatments were applied to system as below:

i) Constant mild water stress: plant were irrigated daily with field capacity (FC) from transplanting until day 2. Hereafter, the amount of daily irrigated water was reduced to 50% FC until harvest,

ii) Intermittent severe water stress: plant were irrigated daily to FC from transplanting to 50% flowering and withhold water was applied until severe wilting symptoms appear. At this time, plants then were irrigated at full capacity (watering to complete restoration of water) at once. On the next day, again plants then treated repeatedly to another severe drought condition (without water for several days until symptoms appeared). This drought condition was applied and repeated until end of experiment,

iii) Control: plant irrigated daily to field capacity (FC) starting from seedling transplanting.

Figure 4. Bell pepper transplanting 7 weeks after sowing.

3.4 Plant health factors

The dominant pest and disease during the experiment were aphids and whiteflies. As integrated pest management program to control the whitefly, an Enforce© (Bioforce Limited, NZ) tag was put inside the glasshouse. However, when the number of pest and disease attack increased, chemical sprays were applied according to Massey Horticultural Unit procedures. A restricted area sign was erected in order to ensure the health and safety. No disease issues arose in this experiment.

3.5 Treatment and plots

Each plot consisted of four plants and each block comprised 24 plants (3 irrigation treatments x 2 varieties x 4 plants per treatment). Each plant was fed through a drip irrigation system. Making total 96 experimental plants (Figure 5). A guard plants were place surrounding the experimental plant.

VS0	CS0	CS0	VS2		CS1	VS2	CS2	VS2
VS0	VS1	VS0	CS1		VS1	CS2	CS1	CS0
CS2	CS0	VS1	CS2		VS0	CS1	VS2	VS1
CS1	VS2	CS1	VS1		VS0	CS2	VS2	CS0
VS0	CS0	VS1	VS2		CS2	VS0	CS1	VS2
CS2	VS1	CS1	CS0		VS0	VS1	CS2	CS0
VS1	CS0	VS2	VS0		CS2	VS2	VS1	VS0
CS0	CS1	VS1	CS1		VS0	VS1	CS1	CS2
VS2	VS0	CS0	CS2		CS0	CS1	CS2	VS2
CS1	VS2	CS0	CS2		CS2	CS0	VS0	VS1
VS2	CS1	VS2	VS0		CS1	VS2	CS2	VS1
CS0	VS0	CS2	VS1		VS1	CS0	VS0	CS1

Figure 5. Experimental layout in the glasshouse (V: Viper, C: Cupra, S0: Control, S1: Constant mild stress and S2: Intermittent severe water stress.

3.6 Data collection

There were some measurements and observation investigated during plant growth and yield harvested in this experiment, namely:

3.6.1 Observation and measurement in plant vegetative growth

1. Plant height

   Plant height was observed every week until 17 weeks after transplanting (WAT). The measurement was taken by using a tape measure from the base of plant until the highest tip of the plant.

2. Plant weight

   Plant fresh weight and dry weight basis observed at the end of experiment. All leaves was removed from the stem and weighted separately to get fresh weight. Dry weight measurement was recorded after the leaves and stem dried by oven with 70°C temperature for 48 hours. Total dry weight was the total from leaves and stem.

3. Number of nodes per plant

   Number of nodes per plant was observed at 17 weeks after transplanting (WAT) by counting the total number of nodes from two main stem and divided by two.

4. Leaf Area (LA)

   After leaves were removed and cleaned from sap and dust, the leaf was flattened. Leaf area measurement were done by placed the leaf samples one by one for every plant between the guides on the lower transparent belt and allowed to pass through the Li-3100 area meter. Object width is scanned by camera system to give length information and presented on the light emitting diode (LED) display. The final reading after all the leaf sourced from one plant completed were recorded.

3.6.2 Media moisture content and physiological response measurement:

1. Media moisture content (MMC)

   The media moisture for all treatments was recorded four times using Time Domain Reflectometry (TDR; MiniTrase TDR) when plant on intermittent severe treatment showed wilting symptom. Fifteen-centimetre probe from

TDR was penetrate media near the stem for 30 seconds. This measurement was done on every pot, started from 11.00 am.

2. Photosynthetic rate (PR) and Stomatal conductance (SC)

   Instantaneous gas exchange measurements were made on four fully expanded leaves in the upper part of the canopy for each variety and treatments between 9:00 am and 3:00 pm using an open gas exchange system (LI-6400; LI-COR, Inc., Lincoln, Nebraska USA). Measurements of net CO_2 assimilation (A), stomatal conductance (gs), and transpiration (E) were performed at saturating red light (1000 μmol m^{-2} s^{-1}) achieved with the red LED lamp of the system, with an additional 10% of blue light to maximize stomatal opening, and 400 μmol CO_2 mol^{-1} in the cuvette. Air temperature and humidity in the chamber was set to match environmental conditions, in consequence of which leaf temperature ranged between 28 and 34 °C depending on leaf water status.

3.6.3 Observations in yield quantity and quality:

1. Yield (fruit production)

   Fruits were harvested after 85 % to 90 % red colour appear to ensure yield quality fruit weight and number of fruits per plant were recorded (Calpas, 2004). At this stage, number of non-marketable fruit was also recorded, such as deformed or blossom end rot incidence. The marketable fruit in this experiment is stated as a combination of approved diameter (medium to extra-large) and the absence of blossom end rot.

2. Number of fruits produce per plant

   The fruits produce per plant were recorded by counted the number of fruits which has been harvested.

3. Fruit length and diameter

   At maturity, three fruit were selected from every experimental plant in order to determine its length and diameter. Measurements will be taken on the widest and longest part of the fruit with an electronic Vernier calliper (Model 50-321, Mitutoyo, Japan).

4. Total soluble solids (TSS)

   Fruit were cut in the equatorial area and sliced into small portions. A garlic press were used to extract the juice. An electronic refractometer (PAL 1, Atago Japan) were calibrated to zero with distilled water. Several drops of clear juice were put onto the refractometer's prism. After each run, the prism was cleaned with distilled water and tissue paper, to avoid any contamination.

5. Fruit firmness

   The measurement of fruit firmness is done by using Efigi stand penetrometer. Fresh harvested fruit was assessed in the middle (equator) part of fruit. Every sample fruit was pressed two times in the opposite position. Total reading was divided by two.

6. The incidence of Blossom-end rot

   Observation were made during each harvest for Blossom end Rot (BER) incidence. The incidence of the physiological disorder was calculated as percent fruits giving evidence of BER.

3.7 Experimental site

The plants were grown in a media in a glasshouse at the Massey Horticultural Unit (MHU) Massey University, Palmerston North, New Zealand. (Latitude 40° 19' South, longitude 174° 46' East, altitude 25 m above sea level).

3.8 Statistical analysis

All data were recorded in Excel, and statistical analysis was performed using both Excel and IBM SPSS statistic 24. Normality assumptions were tested before analysis of variance (ANOVA) and significant results were statistically compared. Tukey test was used for total leaf area, plant fresh weight, plant dry weight, number of nodes, number of fruits per plant, weight of fresh fruit and weight of dry fruit.

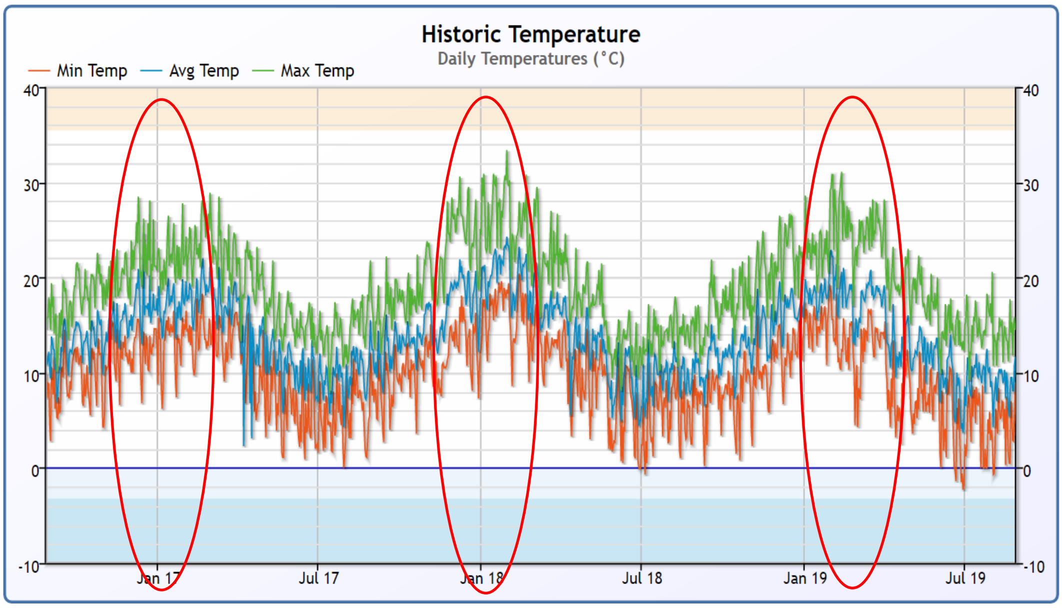
The Chi square test was used to examine the relationship of irrigation for marketable fruit. T-test was used for number of total fruits harvested.

CHAPTER IV

Results and Discussion

4.1. Environmental condition (glasshouse temperature during study)

Comparing with three years' data captured from the Palmerston North weather (source**:** http://palmyweather.co.nz/trendshistoric)**,** the summer weather in 2017 (temperature points around January 17) had more low temperature below 10°C and the warmest temperature was below 30°C. There was an increasing maximum temperature in summer or January 2018 to around 30-31°C, but there were still some days with the lowest temperature at below 10°C. Meanwhile in 2019, there were increasing in the lowest temperature during summer in average to above 12°C even though the maximum temperature was initially high in early January, but still lower than summer in 2018, in average (Figure 6).



Source: http://palmyweather.co.nz/trendshistoric.php

Figure 6. Temperature data from January 2017 – July 2019.

Temperature records were maintained inside Greenhouse 25 (GH25) from 1st November 2017 to 28th February 2018. The greenhouses were intended to operate in the range 18-25 °C using controlled heating and ventilation. In fact, the highest recorded temperature was 36°C on 29th January 2018 and the lowest temperature was 13.5°C on 21 December 2017. The average temperature during the experiment was 24.08°C (Figure 7).

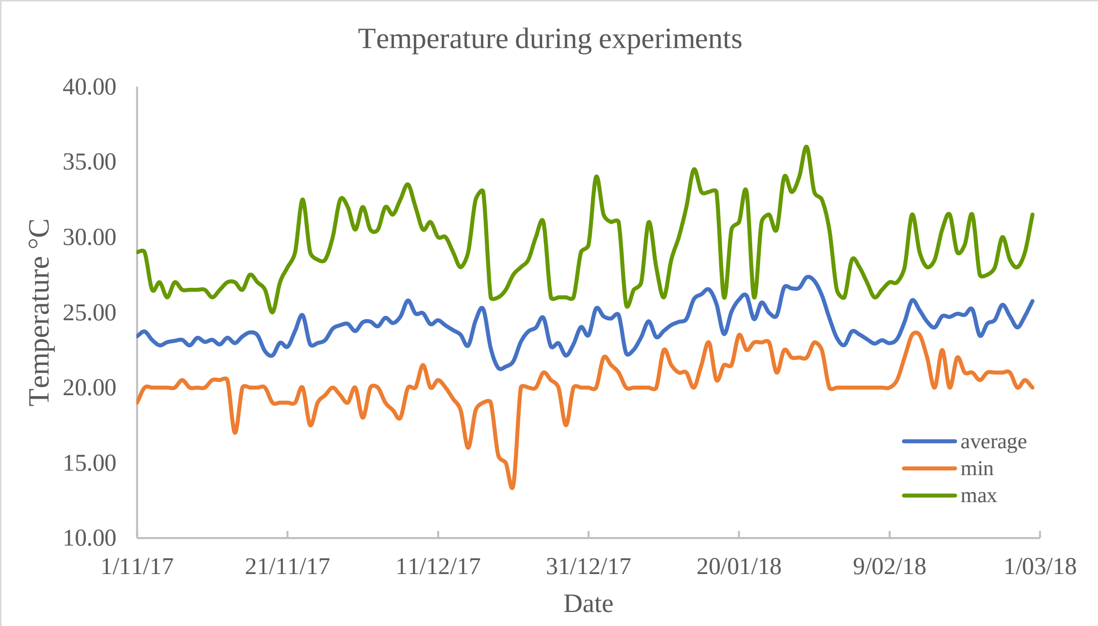


Figure 7. Temperature fluctuation in the glass house during experiment.

Even though bell pepper requires warm temperature to grow optimal, bell pepper need an optimum temperature between 22-28°C during daytime and 18°C at night. This experiment had higher temperature either at night or daytime, with temperature difference from maximum (above 30°C) to the lowest (mostly around 20°C in average). The warmer condition during cultivation than what we expected may influence especially plant production which according to Rylski & Spigelman, (1982) higher temperature will increase flower and fruit abortion on bell pepper.

4.2 Seed Germination

Two bell pepper varieties, Cupra and Viper, were sown on 12th September 2017. Cupra germinated earlier than Viper (Table 5), consistent with the early maturity characteristics of this variety (Supplementary Table). However, both species showed a high percentage germination by 14 DAS and produced healthy seedlings (Figure 8). Each seedling was then transferred to a 10 L pot at 7 weeks (1 November 2017) after sowing for their water (stress) treatment (Figure 9).

Table 5. Seed germination percentage at 8 and 14 day after sowing (DAS)

Variety	8 DAS	14 DAS
Cupra	78 %	88 %
Viper	5 %	95 %

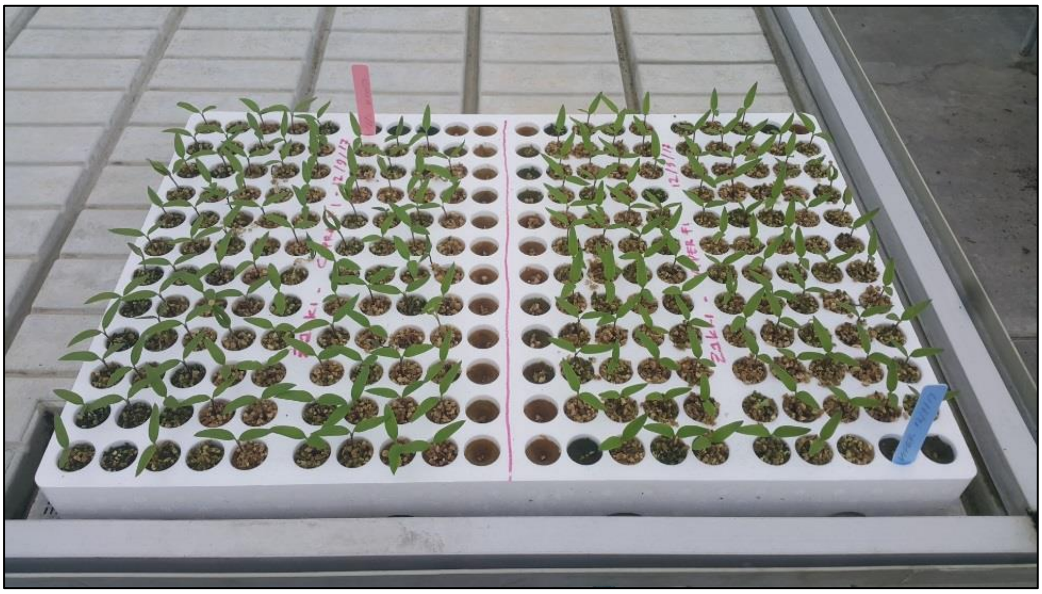


Figure 8. Bell pepper germination 14 days after sowing.

Figure 9. Bell pepper in the green house at 7 weeks after sowing.

4.3 Media moisture content

The percentage of media moisture content was measured during vegetative growth, on four recording dates. The pattern of media moisture content of each treatment was similar for both varieties, ensure treatments were at the same condition and comparable for both varieties.

Media withhold water with similar pattern for both control and constant mild water stress, showing media water content increased from first recording date to the last due to daily watering. In the control treatment, plants were irrigated daily to field capacity while at constant mild water stress, plants were daily irrigated with half field capacity (50%) of control since day 3 after transplanting into greenhouse (3 November 2017) until end of experiment (15 March 2018). Constant mild treatment lost about 5% media moisture content compared to control (Figure 10).

On the contrary, media moisture content in intermittent severe water stress treatment was very low (below 20% on every observation date). In this treatment, plants were irrigated daily to FC from transplanting to 50% plants flowering, with assumption that plants were generaly in generative phase. Hereafter, withholding water was applied until severe wilting symptoms appear. The irrigation for this treatment applied after the media moisture reading on wilting plant were done, and there were 4 times for watering at 1 Dec, 10 Dec, 18 Dec and 28 December.

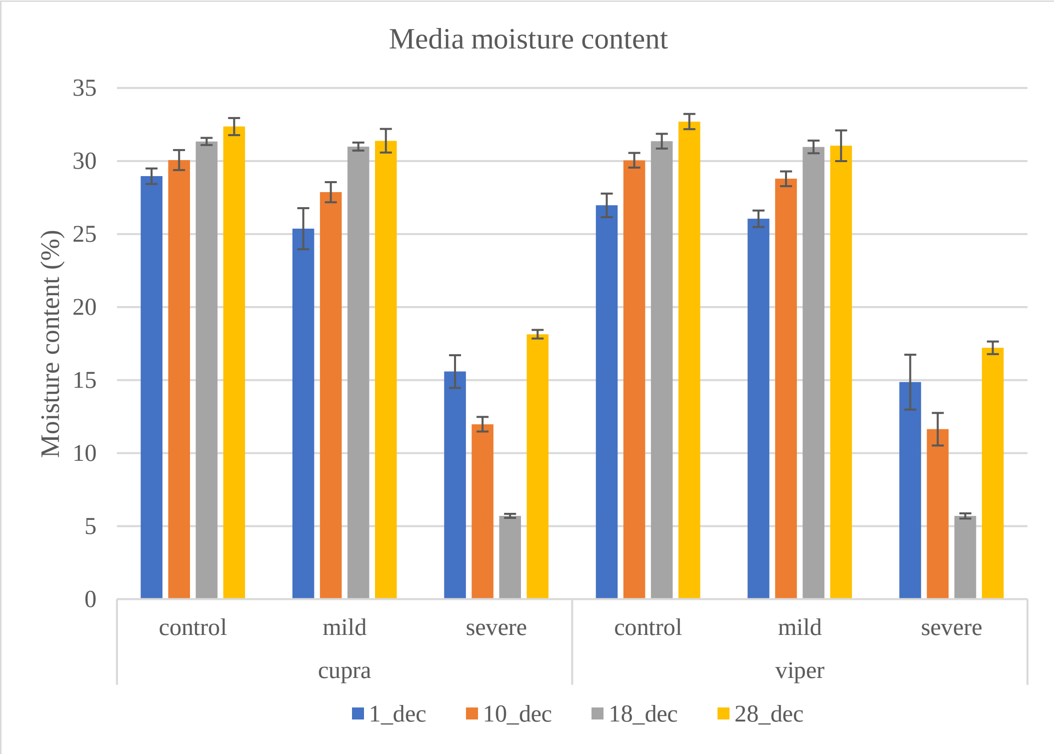


Figure 10. Media moisture content (%).

Severe wilting symptom (Figure 11) appears as plants morphological response to water stress. At this condition, plants were irrigated to full capacity (watering to complete restoration of water) at once. Plant visibly recovered within four hours after re-watering.

Media moisture content (Figure 10) in intermittent severe water stress treatment was very low (below 20% on every observation date). By the 1st of December, the media moisture content in intermittent severe water stress treatment was about half of the control treatment, for both varieties Cupra and Viper. The TDR measurement after 10 days gave near one third available water content reading compared to control and constant mild water stress treatment. The next measurement was taken eight days after and the moisture reading was only one sixth for intermittent severe water stress compared to other two treatments. The lowest available moisture content in this experiment was 5% and at this stage, intermittent severe water stress caused significant changes in plants growth.

Figure 11. Plant with severe wilting symptoms.

Media in the pot was very dried out and lead the whole plant leaves wilting or showing severe drought symptoms. With high temperature during daytime in the greenhouse, available water in media apparently evaporated and media lost its moisture content faster. Lack of water within the media create a water stress condition within the plant and severe wilting symptoms appeared as plant adaptation to water deficit (Jones, 2013). At this condition, plants then re-watered to the field capacity and based on observation, plant visibly recovered within four hours after re-watering.

4.4 Vegetative growth

Plants were placed into greenhouse on November 1st, 2017 and observation on plant vegetative growth were made for around 4 months on a weekly basis. At the end of the experiment (15 March 2018), total leaf area (cm^2) and plant vegetative parts (stem and leaves) as fresh and dry weight were measured including number of nodes at the final harvest (Table 6).

Katerji et al. (1991) reported that drought stress reduced leaf area while plant increased leaves density. There was also reduction on dry mass (up to 23%) due to watering tomato plant in half capacity during cultivation (Zegbe et al., 2006). Moreover, González-Dugo et al., (2007) supported those previous findings that drought stress during plant growth and development stages on bell pepper reduced leaf area, as well as decreased plant fresh and dry weight.

It was found that reducing water input during plant growth and development decreased plant (stem and leaves) water content (%) and extending drought period by intermittent severe treatment also reduced both plant fresh and dry weights for those two varieties in this experiment. However, no significant difference on total leaf areas and number of nodes between treatments were found (Table 6). Recurrent water stress affected plant growth (both fresh and dry weight), while application of half water capacity in constant mild water stress did not significantly affect plant growth or plants in this treatment were tolerant during vegetative phase.

Table 6. Total leaf area (cm^2), plant weight (g), water content and number of nodes of two bell pepper varieties with water stress treatments during cultivation in the screen house.

Varieties	Treatment	Total leaf area (cm^2)	Plant Fresh weight (g)	Plant Dry weight (g)	Water content (%)	Number of nodes
Cupra	Control	3172.48±301.52 ^{ns}	301.16±9.45 ^{ab}	56.73±1.65 ^{ab}	81.16	18.66±0.3^{ns}
	Mild	2801.66±198.89 ^{ns}	295.34±11.47 ^{ab}	56.65±2.46 ^{ab}	80.82	18.75±0.35 ^{ns}
	Severe	2313.60±161.15 ^{ns}	245.11±8.26 ^{b}	48.08±1.62 ^{b}	80.38	17.06±0.38 ^{ns}
Viper	Control	2427.66±226.96 ^{ns}	296.60±22.84 ^{ab}	55.95±4.41 ^{ab}	81.14	18.84±1.33 ^{ns}
	Mild	3091.06±167.40 ^{ns}	329.91±14.39 ^{a}	65.44±2.99 ^{a}	80.16	19.66±0.73 ^{ns}
	Severe	2879.31±163.04 ^{ns}	266.56±13.19 ^{b}	53.37±2.99 ^{b}	79.98	17.91±0.45 ^{ns}

Mean value ± standard error. Different letter within column indicate significant difference at $P<0.05$ (Tukey test).

4.5 Plant physiological responses

Furthermore, stomatal conductance (Gs), photosynthesis rate (PN), transpiration (E), internal carbon (CI) and vapour pressure deficit (VPD) were assessed to confirm plant physiological responses during this experiment (Table 7). To compare how plants responded either on watering or drought stress, two observations, at 5/12/2017 and 20/12/2017, were made when plants looked healthy and unwilted, while the other two observations were made during appearance of wilting symptoms (prior to re-watering).

From these observations, significant plant physiological responses to drought stress and adaptability to adjust during normal condition (after watering) were found. Significant decrease on transpiration only happened once in 13/12/2017 on severe water stress, thus known as adaptive strategy to increase water use efficiency during water stress as described by Tardieu (2013). Reduction on stomatal conductance, reduction on photosynthesis rate and reduction on transpiration are the most significant activities found during restricted water condition, both in constant mild water stress and intermittent severe water stress. These findings (reduction on plant physiological activities) showed plants ability to adapt in drought conditions. Mechanisms such as stomatal enclosure, decreasing transpiration and photosynthesis activities were plant tolerant mechanisms (Chaves & Oliveira, 2004) to reduce cellular water loss (Schulze, 1986) and enable plant to survive during stress condition.

More specifically, at the end of observation (30/12/2017), genetic-specific differences between bell pepper varieties in this trial were suggested to cause difference in physiological adaptation mechanisms under water scarcity condition. Cupra variety had higher stomatal conductance and higher photosynthesis rate, thus physiologically less sensitive to water stress than Viper. These two physiological responses might explain the plant’s adaptation mechanisms to survive on drought condition, as described in Tardieu (2013).

Table 7. The effect of irrigation under constant mild water stress (half water capacity), intermittent severe water stress and control (full water capacity) treatments on stomatal conductance (Gs, mmol m^{-2} s^{-1}), photosynthesis rate (PN, µmol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), internal carbon (CI, µmol mol^{-1}) and vapour pressure deficit (VPD, kPa) on two bell pepper varieties.

Dates	Factor		Gs	PN	E	CI	VPD
5/12/2017	Variety (V)	Cupra	370.4^{NS}	14.5^{NS}	14.1^{NS}	281^{NS}	4.3^{NS}
		Viper	381.4	14.4	14.1	282.4	4.2
	Irrigation (I)	Control	402.7^{NS}	15.2^{NS}	14.5^{NS}	281^{NS}	4.1^{NS}
		Mild	376.5	14.8	14.3	279.3	4.3
		Severe	348.5	13.3	13.4	284.8	4.3
	VxI		NS	NS	NS	NS	NS
13/12/2017	Variety (V)	Cupra	271.7^{NS}	17.7*	5.4^{NS}	248.8^{NS}	2.3^{NS}
		Viper	287.4	16.1	5	234	2.2
	Irrigation (I)	Control	417.6*	19.2*	6.5*	259.7*	2.5^{NS}
		Mild	247.9	15.5	5.2	246.8	2.3
		Severe	173.2	15.9	3.9	217.6	2
	VxI		*	*	*	NS	NS
20/12/2017	Variety (V)	Cupra	375.7^{NS}	15.1^{NS}	6.1^{NS}	265.8^{NS}	2.1^{NS}
		Viper	357.713	13.7	5.7	270	2.2
	Irrigation (I)	Control	435^{NS}	15.6^{NS}	6.3^{NS}	273.3^{NS}	2^{NS}
		Mild	371.7	14.1	6.1	273.7	2.1
		Severe	293.4	13.4	5.2	257.3	2.2
	VxI		NS	NS	NS	NS	NS
30/12/2017	Variety (V)	Cupra	509.6*	20.2*	8.2^{NS}	285.1^{NS}	1.9^{NS}
		Viper	429.9	18.8	7.5	278.1	2
	Irrigation (I)	Control	520.9*	19.9	8.2^{NS}	286.5	1.9^{NS}
		Mild	474.9	19.6	8.1	284.5	2
		Severe	413.4	19	7.4	273.8	2.1
	VxI		*	NS	NS	*	NS

NS: non significant, or *: significant different at $P < 0.05$.

4.6 Fruit production: yield and quality

By the end of December 2017, fruits from both bell pepper varieties, Cupra and Viper, were ready to harvest. Fruits were harvested from end of December to mid-March 2018 and t-test of number of total fruits in every treatment showed not statistically different among either those two varieties or irrigation treatments (Table 8).

Table 8. Number of total fruits harvested from end of December 2017 to mid-March 2018.

Treatment	December		January		February		March	
	Cupra	Viper	Cupra	Viper	Cupra	Viper	Cupra	Viper
Control	1	2	86	62	25	31	67	64
Constant Mild	3	3	77	68	22	24	81	83
Intermittent Severe	3	3	75	54	23	37	86	77
Grand Total	7	8	238	184	70	92	234	224

Number of plants fruiting were very low by end of December 2017 and complete yielding was unable to collect on February 2018 due to technical issue. Thus, harvest data from both December and February were not included for further analysis. Moreover, there was also no significant results on number of fruits produce per plant at either different treatments or varieties in this study (Table 9). From this information (Table 8 and Table 9), plants on both varieties showed the effective use of water, to maintain production under limited water supply (Blum, 2009) with no significant difference with control treatment.

Table 9. Average number of fruits produced per plants.

Variety	Irrigation	Number of fruits per plant (mean ± SE)
Cupra	Control	9.56 ±1.23^{ns}
	Constant mild	9.88 ±0.55 ^{ns}
	Intermittent severe	10.06 ±0.48 ^{ns}
Viper	Control	7.88 ±0.81^{ns}
	Constant mild	9.44 ±0.57 ^{ns}
	Intermittent severe	8.19 ±0.41 ^{ns}

Data shown are mean value ± standard error. We did not find any significant difference at P<0.05 (Tukey test). ns: statistically not significant.

Even though plants with constant mild water stress treatment showed a good vegetative growth during cultivation (Table 6), there was no significant differences in fruit yield for all treatments (Table 8 and 9). More observation on fruits weight showed that constant mild water stress produced less fresh-fruits mass on Viper variety (Table 10). To be more specific, there was significant interaction between varieties and irrigation treatments on fresh and dry fruits weight. Viper constantly yield higher fresh and dry fruit mass compare to Cupra. Intermittent severe water stress did not significantly different to control on both varieties, but constant mild water stress significantly reduced fresh fruits on Viper.

Table 10. Weight of fresh and dry fruit (grams) per plants.

Variety	Irrigation	Fresh Fruit (mean ± SD)	Dry fruit (mean ± SD)
Cupra	Control	101.31 ± 23.33a	10.72 ± 2.76a
	Constant mild	106.32 ± 25.09a	11.03 ± 5.61ab
	Intermittent severe	108.44 ± 21.04a	11.68 ± 3.22ab
Viper	Control	146.03 ± 29.08c	14.71 ± 5.50cd
	Constant mild	126.59 ± 29.93b	13.31 ± 6.05c
	Intermittent severe	143.55 ± 20.12c	16.22 ± 6.82d

Data shown are mean value ± standard deviation. Different letter within column indicate significant difference at P<0.05 (Tukey test).

Observations on marketable yield quality showed that constant mild water stress also produced the lowest total of normal fruits and at the same time the highest incidence of blossom end rot compared to control and intermittent water stress

(Figure 12). This condition may be explained that plant showed ability to endure water stress at seedling or vegetative stage but not in generative stage (Ferrara et al., 2011). However, later Díaz-Pérez (2014) found similar fruit production to control at this condition (watering at half field capacity). More studies also found that reduction on water supply in long time had more detrimental effect than completely withholding water supply in short duration (Techawongstien et al., 1992b), thus reducing yield production and quality (Ferrara et al., 2011; Techawongstien et al., 1992b).

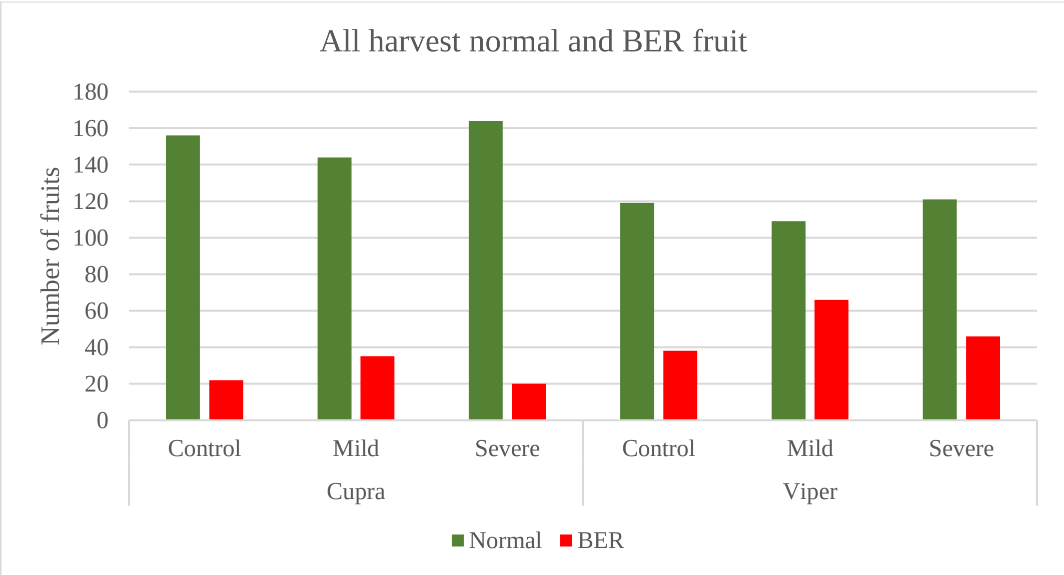


Figure 12. Number of normal fruits, and fruits with appearance of blossom end root harvested from every treatment on Cupra and Viper varieties.

Previous results on plant physiological activities in this study also showed variety or genetic-specific tolerance in this study (Table 7). Viper plants were more responsive to water stress compared to Cupra. Moreover, yield quality from this experiment showed that Cupra variety produced more normal fruits (Figure 12, Table 11) but less weight (Table 10) than Viper. Thus, this finding showed that there was relationship between genetic, plant physiological response to water stress and fruits quality (Sato et al., 2003).

Table 11. Incidence of blossom end rot (BER) on fruits observed.

Variety	Irrigation	Incidence of BER			
		No	%	Yes	%
Cupra	Control	157	87.71	22	12.29
	Constant mild	147	80.77	35	19.23
	Intermittent severe	166	88.77	21	11.23
Viper	Control	119	74.84	40	25.16
	Constant mild	110	61.80	68	38.20
	Intermittent severe	123	72.35	47	27.65

Here, marketable fruits were categorized based on number of normal fruits (without BER) and fruits size (medium to extra-large). Despite to the facts that Cupra produced more normal fruits than Viper, further observation in fruit quality in term of fruit size showed that Cupra produced smaller fruits more than Viper (Table 12). Over half of Cupra fruits was in medium grade, while Viper produced more large size and extra-large grades fruits.

Table 12. Fruit grading by sizes.

Treatment	Extra Large (%)		Large (%)		Medium (%)		Small (%)	
	Cupra	Viper	Cupra	Viper	Cupra	Viper	Cupra	Viper
Control	2	22	18	46	70	30	10	2
Constant Mild	4	15	16	36	61	44	19	5
Intermittent Severe	1	25	16	46	74	27	9	2

According to Kirda, (2002), high yielding varieties are more sensitive to water stress than low yielding varieties, and drought condition substantially increased the proportion of unmarketable pepper fruits, in term of fruit size and blossom end root percentage. Thus, genetics or varieties developed different responses to drought conditions (Chaves & Oliveira, 2004) which influenced fruits quality. It is also interesting to assess if there were any response on marketable fruits due to watering condition in every variety based on harvesting time (Table 13). From this analysis, it was found that different irrigation treatments (control, constant mild water stress, and intermittent severe water stress) was significantly affecting fruit quality (marketable fruits) in every variety (except not significant on Viper on January).

However, there were no significant difference on total soluble solid and fruits firmness found between treatments in this study (Table 14).

Table 13. Marketable fruits.

Chi-square test	Marketable fruits on January		Marketable fruits on March	
	Cupra	Viper	Cupra	Viper
Probability	0.010	0.194	0.001	0.006
Chi-count	9.234	3.278	13.998	10.160
Chi-table	5.991	5.991	5.991	5.991
Results	*	NS	*	*

Results: * indicating chi count > chi table (irrigation affected marketable fruit), NS indicating chi count < chi table (irrigation did not affect marketable fruit).

Table 14. Fruit total soluble solid and firmness.

Varieties	Treatment	Total soluble solid (brix)	Firmness
Cupra	Control	6.58 ±0.950	2.672 ±0.394
	Constant mild	6.41 ±1.071	2.709 ±0.564
	Intermittent severe	6.91 ±1.254	2.762 ±0.322
Viper	Control	6.13 ±1.030	2.287 ±0.486
	Constant mild	6.29 ±1.252	2.478 ±0.435
	Intermittent severe	6.29 ±1.041	2.319 ±0.494

Data shown are mean value ± standard deviation. Two way ANOVA showed no significant difference between treatments.

CHAPTER V

Conclusions and Future Recommendations

Water stress treatments had less impact on plant vegetative growth. Yet, plant physiology activities explained bell pepper plants adaptability to tolerate in drought conditions on either mild or intermittent severe water stress in this study. Severe water stress reduced plant physiological activities, and the Viper variety was more sensitive to water stress than the Cupra variety.

Plant adaptability to limited water conditions indicating the effective use of water (EUW)**.** Reducing water supply did not affect plant yield in general (number of fruits), but variety play significant role in marketable yield quality fruits. Even though water stress treatment did not affect fruit weight on Cupra, Viper produced more fruits mass (both fresh and dry weight) and intermittent severe water stress reduced fresh fruits weight on this variety. Moreover, withholding water up to half water capacity found to have more detrimental effect to marketable fruits quality, in term of number of normal fruits (related to incidence of blossom end root) and fruit size. Cupra variety produced more normal fruits, but smaller fruit size than Viper. Furthermore, constant mild water stress decreased number of marketable fruits (large and extra-large fruit size) on Viper compared to other treatments.

Based on these results, it can be concluded that plants experiencing the limited water supply in the severe condition still tolerate the intermittent severe water stress, by considering plant wilting symptoms as an important indicator of stress condition. No significant number of fruits produced per plant between all treatments may indicated potential transforming approach to the production of capsicum and saving of water, thus need further research to confirm this results.

At cellular level, plant wilting symptoms is a manifestation of disruption on water content and turgor. It had been well explained in the literature that those conditions were caused by increasing soluble cellular concentration (cytosol and extracellular matrices), likewise commonly found with accumulation of ABA and osmolytes (i.e.

proline). Continuous severe stress may inhibit plant growth and development, including reproduction, alter plant cell wall elasticity, disruption of homeostasis and ion distribution in the cell. Prolonging this condition may generate critical injury and end up with plant deterioration due to irreversible damage.

Plant mechanisms to adapt in water stress were genetic-specific. Viper variety showed more adaptable physiological response to water stress, thus produced better fruits quality (in term of fruits size/grade and fruits weight) than Cupra. Here, Viper is suggested more tolerant and has better drought tolerant mechanisms to utilize plant sources from photosynthesis to develop better fruits quality instead of quantity, thus has more value (price to weight ratio) that will benefit small farmers than Cupra variety.

Based on this experiment it is clear intermittent severe water condition could be recommended as a screening method to assess and determine adaptive bell pepper plant varieties that efficient in water use. However, further trials is still needed to validate this results on specific water scarcity conditions in the field condition, especially to be adapted as sustainable water efficiency in limited water supply areas in Indonesia. Further observations on root behaviour during drought condition is also strongly suggested and crucial to determine bell pepper plant mechanism toward water scarcity, as one of indicator on plant morphological response and adaptation to water stress.

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Appendix. Description of capsicum variety

Description of Cupra

Fruit type	: Blocky, 3-lobed
Weight (g)	: 185-195
Young/mature fruit color	: green/red
Disease resistance	: high resistance: Tm: 0-2

Strong against blossom end rot (BER)

Generative plant type

Early maturity

Suitable for heated growing environments

Description of Viper

Fruit type	: Blocky, 4-lobed
Weight (g)	: 210-220
Young/mature fruit color	: green/red
Disease resistance	: high resistance: Tm: 0-2

Strong against micro cracking, shoulder cracking and blossom end rot (BER)

Generative plant type

Medium maturity

Suitable for heated growing environments